Myocardium protection against ischemic-reperfusion injury by nitric oxide supplied to the extracorporeal circulation line during cardiopulmonary bypass (experimental study)

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Aim The study was designed to assess the efficiency of exogenous nitric oxide supply to the extracorporeal circulation line for myocardial protection against ischemic-reperfusion injury in acute myocardial infarction simulation during cardiopulmonary bypass in the experiment.

Methods Acute ischemia was simulated in rabbits (n = 20) with subsequent myocardial reperfusion. All animals were anesthetized and mechanically ventilated through a nasotracheal tube. The experiment included occlusion of the left coronary artery by its clamping with a ligature for 45 min with subsequent reperfusion for 120 min during cardiopulmonary bypass. All animals were divided into 2 equal groups: 10 rabbits received nitric oxide supply to the extracorporeal circulation line in a dose of 40 ppm throughout the entire cardiopulmonary bypass period (the main group); 10 rabbits made up the control group. The ratio of the infarction area to the risk area was determined, and the quantity and nature of ventricular arrhythmias were accessed.

Results It was established that nitric oxide supply through the extracorporeal circulation line during cardiopulmonary bypass had a pronounced infarct-limiting effect, the infarct area to the risk area ratio decreased by 15% as compared to the control group, p = 0.0002. There was also a significant antiarrhythmic effect. A lesser quantity of polytopic and polymorphic ventricular extrasystoles were observed in the main group during periods of ischemia and reperfusion (p = 0.003 and p = 0.012). A statistically significant decrease in the venoarterial gradient of the partial pressure of carbon dioxide in the main group was associated with an increase in the urine flow rate amounting to 1.4 [1.3; 1.5] ml/kg/h in the main group and 1.15 [1; 1.3] ml/kg/h in the control group, p = 0.013.

Conclusion The data obtained during the experiment demonstrate the presence of cardioprotective properties of nitric oxide delivered to the extracorporeal circulation line when simulating myocardial ischemic-reperfusion injury. These properties manifest themselves as a 15% decrease of the infarction zone-risk area ratio, a fewer number of arrhythmias and improvement of tissue perfusion during cardiopulmonary bypass. Intraoperative myocardial protection by nitric oxide in patients operated with cardiopulmonary bypass should be the object of further clinical research.

Keywords myocardium protection; preconditioning; ischemic-reperfusion injury; nitric oxide

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Introduction

A cardiac intervention performed under extracorporeal circulation (ECC) is accompanied by diverse changes at all levels of neural reflex regulation, humoral activity and metabolic status [1]. Intraoperative protection of organs and tissues, myocardium in particular, remains one of the challenges in cardio anesthesiology. The incidence of myocardial dysfunction in the early postoperative period varies from 3 to 30% of cases [3] and is determined by a complex interaction of various mechanisms including a special pattern of metabolic processes in the myocardium, free-radical damage, ion paradox and endothelial dysfunction [4].

One of the promising avenues in meeting this challenge is implementation of a preconditioning phenomenon [5, 6]. Ischemic and pharmacological preconditioning is considered to be a powerful method to increase the resistance of the organism to deleterious effects [7–11]. However, due to possible worsening of symptoms and reduced reserves of the myocardium to protect the heart from intraoperative ischemia and reperfusion in cardiac patients, a pharmacologically induced preconditioning is thought of as the most suitable and safe technique [12]. In this case, nitric oxide (NO) launching an infarction-limiting action through activation of cGMP (cyclic guanosine monophosphate)-dependent protein kinase G and formation of free radicals serves as a trigger to adapt the heart to ischemic-reperfusion injury [13–16]. NO synthesis stimulation also plays a key role in mediating a late stage cytoprotective effects of the endogenous organ protection phenomenon [17].

The study was designed to analyze the efficiency of NO delivery to the extracorporeal circulation line to protect the myocardium from ischemic-reperfusion damage when simulating acute myocardial infarction under cardiopulmonary bypass.

Methods

To conduct this prospective pilot study, 20 male rabbits "Soviet chinchilla" weighing 3–3.5 kg were used. All animals were broken down in two equal groups, with 10 of them receiving NO in the extracorporeal circulation line in a dose of 40 ppm during the entire pumping period (main group) and the remaining ten comprised the control group. The animals were kept in a conventional vivarium. All painful procedures and removal from the study were carried out with the animals anesthetized as per the Order of Ministry of Health Care as of April 1, 2016, No. 199н, "On adoption of good lab practice rules", Good laboratory practice principles ГОСТ 33044-2014 and Janet C. Garber, Guide for the Care and Use of Laboratory Animals: Eighth Edition National Research Council, 2011.

The experiment started with mask induction of anesthesia by means of sevoflurane. On achieving the targeted level of anesthesia, retrograde intubation of the trachea was performed in accordance with the technique developed at our center (Patent No. 2611955 as of March 1, 2017) by means of a 2.5 endotracheal tube, with the animal fixed in a supine position. Artificial lung ventilation was done by using Puritan Bennett 760 system (USA) with a 30–40 ml breathing capacity, 50–55 respirations/min in the Controlled Mandatory Ventilation (CMV) mode and the capacity controlled. Anesthesia was maintained with 1.2–1.5 vol.% sevoflurane via Vapor 2000 vaporizer (Drager, Germany).

A standard method of anesthesia monitoring was applied including continuous electrocardiography analysis, invasive arterial pressure monitoring, pulse oximetry, thermometry by Siemens 7000 system (Germany), urination rate registration. To invasively measure arterial pressure and to sample blood for lab analysis, the femoral artery was cannulated with a 20G catheter. The femoral artery was also cannulated with a 20G catheter for infusion therapy. The thermal gauge was placed in the esophagus. The following indicators were monitored: SaO_2 arterial blood saturation, SvO_2 venous blood saturated from extracorporeal circulation line, pCO_2 venoarterial gradient and lactate level. O_2EI extraction index was calculated and the acid-base balance was analyzed. The blood gas content was measured by means of STAT PROFILE Critical Care Xpress device (Nova Biomedical, USA). Blood was sampled at the following stages: before and at the onset of ECC, 45 min of myocardial ischemia, 45 of myocardial reperfusion, 90 min of reperfusion and 120 min of reperfusion. By applying a reflecting photometry method, the above gas analyzer was used to control the level of methemoglobin. For ECC implementation, НПМ-1 pump and a Kids D100 neonatal oxygenator (Dideco, Italy) were applied. The body surface of a

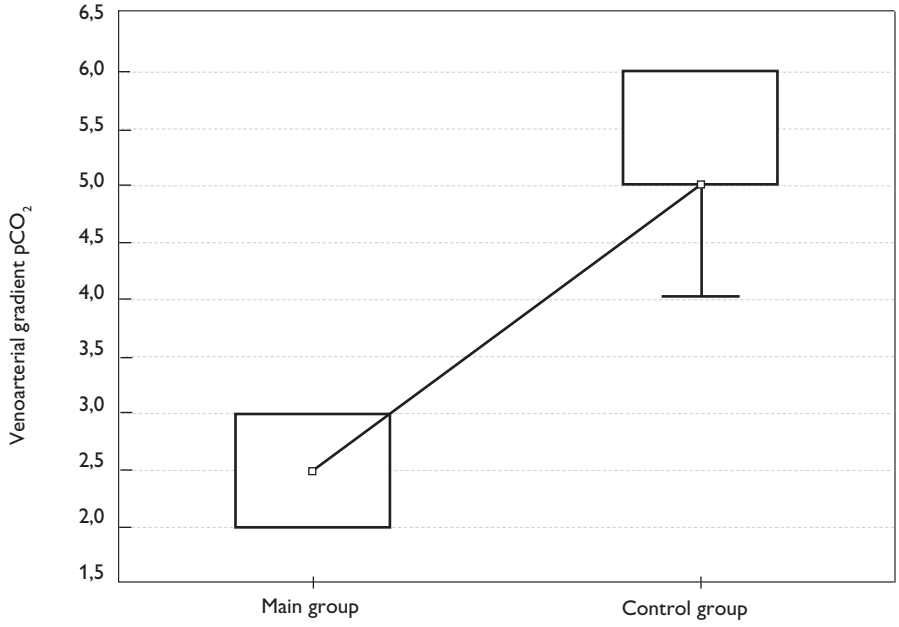

Fig. 1. Venoarterial gradient pCO_2, 45 min ischemia, data are presented as Me [25; 75]; p = 0.0001

rabbit was accepted to be equal to 0.25 m^2. ECC was performed in a pulseless mode, under normothermic conditions. The heart-lung machine was connected according to the accepted "aorta-right atrium" procedure. Perfusion index was 1.8 l/min/m^2. Vasoactive drugs were not used in both groups. The group under study received an additional NO delivery line built in the gas-air main line under aseptic conditions. The NO delivery connector was as close to the heart-lung machine oxygenator as possible and was equipped with a bacterial filter.

After achieving the estimated perfusion rate, NO was delivered into the extracorporeal circulation line in a dose of 40 ppm. PrinterNOX analyzer (CareFusion, USA) was used to monitor NO dosage. The left coronary artery was then occluded by clamping it with a suture for 45 min followed by 120 min reperfusion, with ECC continuing uninterrupted. The NO insufflation protocol underwent no changes during the entire period of extracorporeal circulation.

NO was not delivered to the extracorporeal circulation line in the control group. The experiment included occlusion of the left coronary artery for 45 min followed by 120 min on-pump reperfusion.

Rhythm disturbances in the course of coronary occlusion were recorded in the first 10 min (phase 1) and subsequent 35 min (phase 2), since the mechanisms of arrhythmias differ in these periods. The first 45 min of reperfusion were taken as phase 3. Phase 1 revealed reentry type rhythm disorders, phase 2—ischemia and phase 3 tended to result both in reentry disorders and ectopic automatism [2].

The ratio between an infarction zone and a risk area was determined by means of a modified technique developed by J. Neckar [18]. To evaluate the risk area (hypoperfusion), the suture was retightened, the heart was dyed with 5% potassium permanganate solution administered via the aortic cannula. The heart was then eviscerated from the thorax cavity and the right ventricle was removed from it. 1 mm slices were then cut strictly perpendicular to the longitudinal vertical axis of the heart by using HSRABBIT002-1 slicer (Zivic Instruments, Pittsburgh, USA). The slices were scanned on both sides by means of HP Scanjet

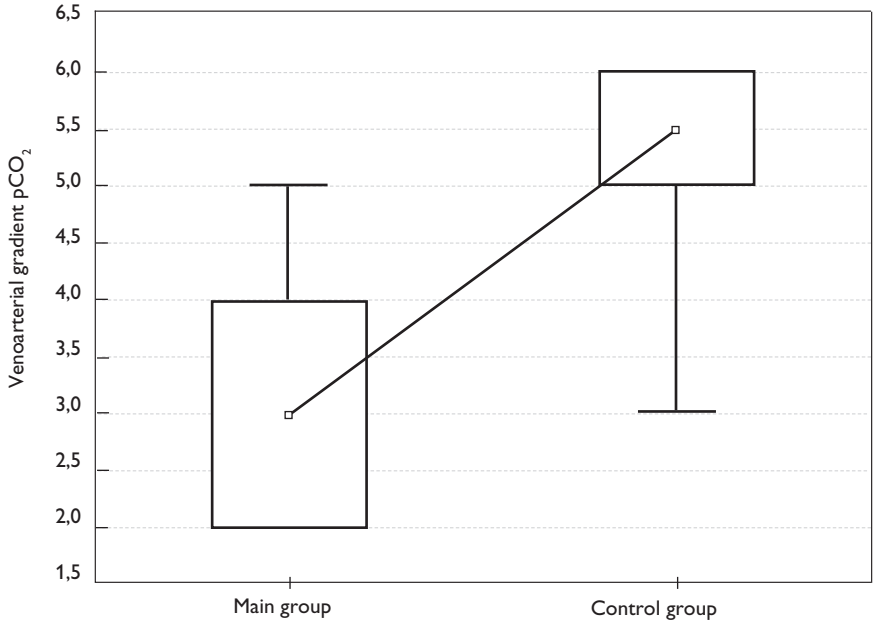

Fig. 2. Venoarterial gradient pCO_2, 45 min reperfusion, p = 0.0016; data are presented as Me [25; 75]

G4050 photo scanner (Hewlett-Packard, Palo Alto, USA) with very high resolution (2400 dpi). The zones with necrotic myocardial tissues were marked off in the hypoperfusion area. A computerized planimetric method based on original software was used to determine infarction zone and risk area values. The computerized planimetric method was also used to estimate the total area of slices, the aggregate area of the infarction zone and risk area for each heart singly. ImageJ2 software was applied to carry out the measurements [19]. The nidus of infarction was determined as a percentage-based ratio between the infarction zone and the size of the risk area.

Statistical analysis

The obtained data were analyzed by using Statistica 10.0 (StatSoft) program. Quantitative values are presented as median (25–75 percentiles). Mann – Whitney U-test (for independent variables) and Wilcoxon T-test (for dependent variables) were used to compare quantitative parameters. A Chi-Square test was used to assess the differences in the incidence of ventricular arrhythmias between the groups. The differences were considered as statistically significant at $p<0.05$.

Results

In both groups the animals demonstrated stable rates of metabolism, acid base composition of blood and ionogram blood test. pH, buffer base levels, concentration of lactate, glucose, ions of sodium, potassium and calcium were within the normal range. When analyzing intergroup differences, a considerable decrease in pCO_2 venoarterial gradient was recorded in the 45 min ischemia (Fig. 1), p = 0.0001, and in the 45 min reperfusion, p = 0.0016 (Fig. 2).

The decrease of pCO_2 in the main group was associated with a significant elevation of the urine flow rate during ECC, which accounted for 1.4 [1.3; 1.5] ml/kg/h in the main group and 1.15 [1; 1.3] ml/kg/h in the control group, p = 0.013 (Fig. 3).

Within the first 10 min of myocardial ischemia in the main group, polytopic and polymorphic ventricular extra systoles were observed rather rarely, p = 0.003. They were more typical for the control group. Ventricular fibrillation developed in 1 animal only. Subsequent 35 min of ischemia in the main group

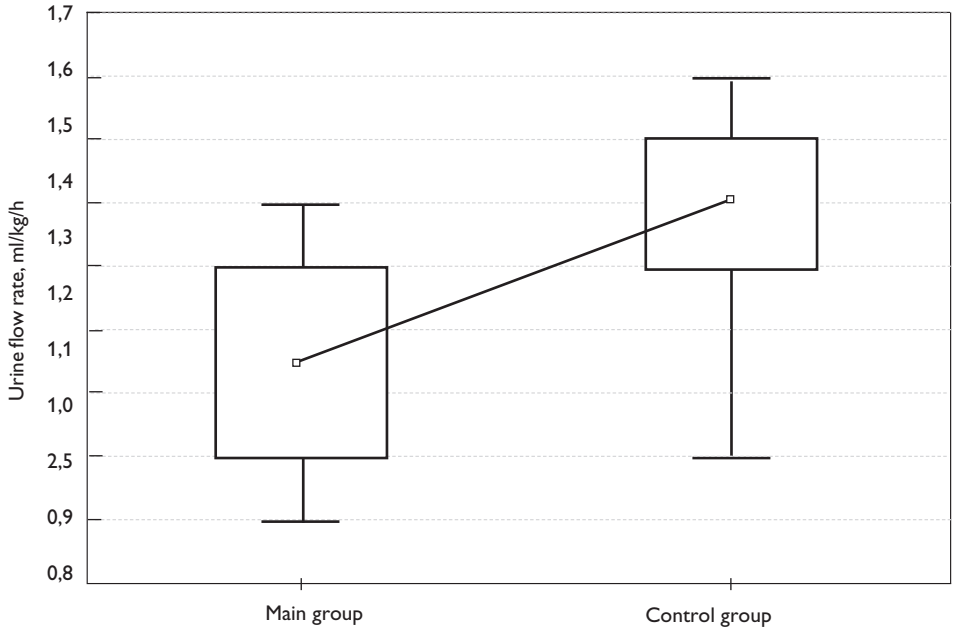

Fig. 3. Urine flow rate, ml/kg/h; p = 0.013

resulted in greater electric stability of the myocardium as compared to that of the control group animals, p = 0.001. During reperfusion various types of ventricular arrhythmias were registered, a statistically significant lesser number of polytopic and polymorphic ventricular extra systole was observed in the NO-protection group, p = 0.012. Ventricular fibrillation in the reperfusion phase developed in 4 animals of the control

Nitric oxide delivery into the extracorporeal circulation line and its impact on arrhythmia occurrence during ischemia and reperfusion

Phase	Arrhythmia	Main group	Control group	p
10 min of ischemia	no ventricular arrhythmia or rare polytopic ventricular extra systole	5 (50%)	6 (60%)	0.5
10 min of ischemia	polytopic and polymorphic ventricular extra systole	2 (20%)	9 (90%)	0.003
10 min of ischemia	ventricular fibrillation	0	1 (10%)	0.5
35 min of ischemia	no ventricular arrhythmia or rare polytopic ventricular extra systole	5 (50%)	6 (60%)	0.5
35 min of ischemia	polytopic and polymorphic ventricular extra systole	1 (10%)	7 (70%)	0.001
35 min of ischemia	ventricular fibrillation	1 (10%)	1 (10%)	1
45 min of reperfusion	no ventricular arrhythmia or rare polytopic ventricular extra systole	5 (50%)	4 (40%)	0.5
45 min of reperfusion	polytopic and polymorphic ventricular extra systole	2 (20%)	8 (80%)	0.012
45 min of reperfusion	ventricular fibrillation	0	4 (40%)	0.04

Pearson Chi-Square test was used. Data are presented as n (%)

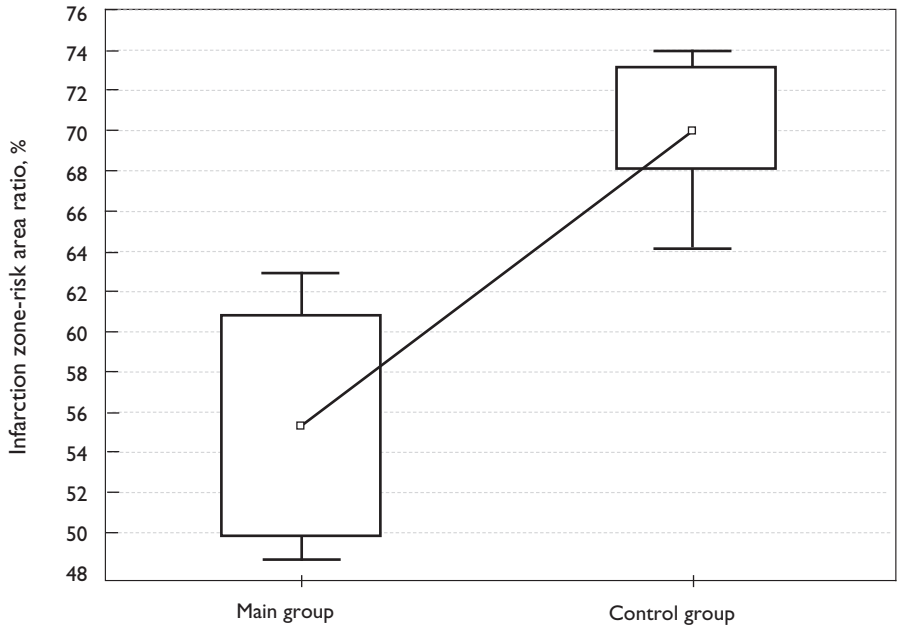

Fig. 4. Infarction zone-risk area ratio, the data are presented in % as Me [25; 75], Mann – Whitney test was used; p = 0.0002

group, while it was not noted in the main group, p = 0.04 (see the Table). The infarction zone-risk area ratio in the main group amounted to 55.6 [50; 61] % which was 15% less than that in the control group—69.9 [68; 73] %, p = 0.0002 (Fig. 4).

Discussion

Nitric oxide is known to participate in implementation of various protective effects of adaptation through a change of the functional state of mitochondria. Possible organ-protective mechanisms include cascades of intercellular transmitters with activation of ATP-sensitive potassium (KATP) channels and inhibition of mitochondrial conductive ostioles as final effectors of preconditioning and reducing mitochondrial injury during hypoxia/anoxia. NO-dependent suppression of adrenergic stimulation and contractility with preservation of endothelial vasodilatation and reduction of calcium overload of the cells tends to decrease the no-reflow phenomenon. The study on rodents shows that nitric oxide inhalation leads to fast accumulation of NO metabolites in blood and tissues, thus contributing to cardiac protection in cases of ischemic-reperfusion injuries and to a decrease of infarction zone-risk area ratio by 31% [20, 21].

The data obtained in our study also point to cardioprotective properties of NO when delivered in the extracorporeal circulation line while simulating ischemic-reperfusion damage of the myocardium.

The study revealed an infarction-limiting effect that reduces infarction zone-risk area ratio by 15% as compared to the control group. Its antiarrhythmic effect is also noteworthy.

Cardiac patients have an increased risk of acute kidney / gastrointestinal tract injury, especially in the case of concurrent surgery [22]. Development of hemolysis due to prolonged ECC and use of donor blood components, as well as limitation of NO-bioavailability lead to microcirculation disorders and systemic aberrations of organ blood flow [23].

Recent studies confirm the safety of NO application during 24 h, as well as a renoprotective effect of nitric oxide when used during ECC and during 24 h after it in the form of inhalation. This effect manifests itself

as alleviation of acute kidney injury following cardiac interventions under prolonged perfusion periods [24].

According to our data, the reduction in the pCO_2 venoarterial gradient in the main group was associated with a significant increase in the urine flow rate during ECC. This indicates a more favorable blood circulation mode in the splanchnic system organs including the kidneys during extracorporeal circulation. Determining an optimal dosing regimen requires further research.

Conclusion

The data obtained during our experiment witness cardioprotective properties of nitric oxide if it is delivered in the extracorporeal circulation line when modeling ischemic-reperfusion damage of the myocardium, which manifest themselves as a decrease in the infarction zone-risk area ratio by 15% and a lesser quantity of arrhythmias. Delivery of nitric oxide in the extracorporeal circulation line tends to improve tissue perfusion during under cardiopulmonary bypass. Intraoperative organ protection of the myocardium with the help of nitric oxide in patients operated under cardiopulmonary bypass should be in focus of further clinical studies.

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The study did not have sponsorship.

Conflict of interest

Authors declare no conflict of interest.

Author contributions

All the authors comply with 4 ICMJE authorship criteria and contributed equally at every stage of the study.

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References

1. Karaskov A.M., Lomivorotov V.V. *Biokhimicheskaya adaptatsiya organizma posle kardiokhirurgicheskikh vmeshatelstv*. Novosibirsk: Geo Publ.; 2004. 288 p. (In Russ.)
2. Maslov L.N., Lishmanov Yu.B., Krylatov A.V., Sementsov A.S., Portnichenko A.G., Podoksenov Yu.K., Khaliulin I.G. Comparative analysis of early and delayed cardioprotective and antiarrhythmic efficacy of hypoxic preconditioning. *Bull Exp Biol Med*. 2014;156(6):746-9. PMID: 24824686. https://doi.org/10.1007/s10517-014-2439-7
3. Yau J.M., Alexander J.H., Hafley G., Mahaffey K.W., Mack M.J., Kouchoukos N., Goyal A., Peterson E.D., Gibson C.M., Califf R.M., Harrington R.A., Ferguson T.B.; PREVENT IV Investigators. Impact of perioperative myocardial infarction on angiographic and clinical outcomes following coronary artery bypass grafting (from Project of Ex-vivo Vein graft ENgineering via Transfection [PREVENT] IV). *Am J Cardiol*. 2008;102(5):546-51. PMID: 18721510. https://doi.org/10.1016/j.amjcard.2008.04.069
4. Carden D.L, Granger D.N. Pathophysiology of ischaemiareperfusion injury. *J Pathol*. 2000;190(3):255-66. PMID: 10685060. https://doi.org/10.1002/(SICI)1096-9896(200002)190:3<255::AIDPATH526>3.0.CO;2-6
5. Murry C.E., Jennings R.B., Reimer K.A. Preconditioning with ischemia: a delay of lethal cell injury in ischemic myocardium. *Circulation*. 1986;74(5):1124-36. PMID: 3769170. https://doi.org/10.1161/01.CIR.74.5.1124
6. Likhvantsev V.V., Moroz V.V., Grebenchikov O.A., Gorokhovatsky Y.I., Zarzhetsky Y.V., Timoshin S.S., Levikov D.I., Shaibakova V.L. Ischemic and pharmacological preconditioning. *Obshhaja reanimatologija = General Reanimatology*. 2011;7(6):59. (In Russ.) http://dx.doi.org/10.15360/1813-9779-2011-6-59
7. Jenkins D.P., Pugsley W.B., Alkhulaifi A.M. Ischaemic preconditioning reduces troponin T release in patients undergoing coronary artery bypass surgery. *Heart*. 1997;77(4):314-8. PMID: 9155608. http://dx.doi.org/10.1136/hrt.77.4.314
8. Wu Z.K., Iivainen T., Pehkonen E., Laurikka J., Tarkka M.R. Ischemic preconditioning suppresses ventricular tachyarrhythmias after myocardial revascularization. *Circulation*. 2002;106(24):3091-6. PMID: 12473557. https://doi.org/10.1161/01.CIR.0000041430.32233.5B
9. Julier K.1., da Silva R., Garcia C., Bestmann L., Frascarolo P., Zollinger A., Chassot P.G., Schmid E.R., Turina M.I., von Segesser L.K., Pasch T., Spahn D.R., Zaugg M. Preconditioning by sevoflurane decreases biochemical markers for myocardial and renal dysfunction in coronary artery bypass graft surgery: a double-blinded placebocontrolled, multicenter study. *Anesthesiology*. 2003;98(6):1315-27. PMID: 12766638.
10. Gross E.R., Gross G.J. Ligand triggers of classical preconditioning and postconditioning. *Cardiovasc Research*. 2006;70(2):212-21. PMID: 16448635. https://doi.org/10.1016/j.cardiores.2005.12.019
11. Wall T.M., Sheehy R., Hartman J.C. Role of bradykinin in myocardial preconditioning. *J Pharmacol Exp Ther*. 1994;270(2):681-9. PMID: 8071859.
12. Lomivorotov V.V., Karaskov A.M. *Remote ischemic preconditioning in cardiac surgery*. Novosibirsk, Geo Publ.; 2010. 127 p. (In Russ.)]
13. Cuong D.V., Kim N., Youm J.B., Joo H., Warda M., Lee J.W., Park W.S., Kim T., Kang S., Kim H., Han J. Nitric oxide-cGMPproteinkinase G signaling pathway induces anoxic preconditioning through activation of ATP-sensitive K+ channels in rat hearts. *Am J Physiol Heart Circ Physiol*. 2006;290(5):H1808-17. https://doi.org/10.1152/ajpheart.00772.2005
14. Lebuffe G., Schumacker P.T., Shao Z.H., Anderson T., Iwase H., Vanden Hoek T.L. ROS and NO trigger early preconditioning:

relationship to mitochondrial KATP channel. *Am J Physiol Heart Circ Physiol*. 2003;284(1):H299-308. PMID: 12388274. https://doi.org/10.1152/ajpheart.00706.2002

15. Xu Z., Ji X., Boysen P.G. Exogenous nitric oxide generates ROS and induces cardioprotection: involvement of PKG, mitochondrial KATP channels, and ERK. *Am J Physiol Heart Circ Physiol*. 2004;286(4):1433-40. PMID: 14656708. https://doi.org/10.1152/ajpheart.00882.2003
16. Bolli R., Dawn B., Tang X. L., Qiu Y., Ping P., Xuan Y.T., Jones W.K., Takano H., Guo Y., Zhang J. The nitric oxide hypothesis of late preconditioning. *Basic Res Cardiology*. 1998;93(5):325-38. PMID: 9833145. https://doi.org/10.1007/s003950050101
17. Kodani E., Xuan Y.T., Takano H., Shinmura K., Tang X.L., Bolli R. Role of cyclic guanosine monophosphate in late preconditioning in conscious rabbits. *Circulation*. 2002;105(25):3046-52. PMID: 12082001. https://doi.org/10.1161/01.CIR.0000019408.67709.B5
18. Neckár J., Papousek F., Nováková O., Ost'ádal B., Kolár F. Cardioprotective effects of chronic hypoxia and ischaemic preconditioning are not additive. *Basic Res Cardiology*. 2002;97(2):5161-7. PMID: 12002264. https://doi.org/10.1007/s003950200007
19. Schindelin J., Rueden C.T., Hiner M.C., Eliceiri K.W. The ImageJ ecosystem: An open platform for biomedical image analysis. *Mol Reprod Dev*. 2015;82(7-8):518-29. PMID 26153368. https://doi.org/10.1002/mrd.22489
20. Mandel I.A., Podoksenov A.Yu., Suhodolo I.V., Podoxenov Yu.K., Svirko Yu.S., Kamenschikov N.O., Mikheev S.L., Sementsov A.S., An D.A., Shipulin V.M., Maslov L.N. Protection of myocardial from ischemic and reperfusion damage (experimental study). *Bull Exp Biol Med*. 2017;164(7):29-34 (In Russ.) Available from: http://www.iramn.ru/journal/bebm/2017/bbm1707.htm
21. Nagasaka Y., Fernandez B.O., Garcia-Saura M.F., Petersen B., Ichinose F., Bloch K.D., Feelisch M., Zapol W.M. Brief periods of nitric oxide inhalation protect against myocardial ischemia-reperfusion injury. *Anesthesiology*. 2008;109(4):675-82. PMID: 18813047. https://doi.org/10.1097/ALN.0b013e318186316e
22. O'Neal J.B., Shaw A.D., Billings F.T. IV. Acute kidney injury following cardiac surgery: current understanding and future directions. *Crit Care*. 2016;20:187. PMCID: PMC4931708. https://doi.org/10.1186/s13054-016-1352-z
23. Vermeulen Windsant I.C., de Wit N.C., Sertorio J.T., van Bijnen A.A., Ganushchak Y.M., Heijmans J.H., Tanus-Santos J.E., Jacobs M.J., Maessen J.G., Buurman W.A. Hemolysis during cardiac surgery is associated with increased intravascular nitric oxide consumption and perioperative kidney and intestinal tissue damage. *Front Physiol*. 2014;5:340. PMID: 25249983. https://doi.org/10.3389/fphys.2014.00340
24. Ignarro L.J., Freeman B. *Nitric Oxide: Biology and Pathobiology*, Third Edition. Academic Press; 2017. 434 p.